Clinical Utility of Serum Endoglin (CD105) in Patients with Hepatocellular Carcinoma

Thesis
Submitted for Partial Fulfillment of M.Sc. Degree in Clinical and Chemical Pathology

By

Sherif Abdelfattah Mahmoud

M.B., B.Ch.
Faculty of Medicine - Ain Shams University

Supervised by

Professor / Nasser Sadek Rezk
Professor of Clinical and Chemical Pathology,
Faculty of Medicine - Ain Shams University

Professor / Manal Abdel Baky Mahmoud
Professor of Clinical and Chemical Pathology,
Faculty of Medicine - Ain Shams University

Professor / Nermine Helmy Mahmoud
Professor of Clinical and Chemical Pathology,
Faculty of Medicine - Ain Shams University

Faculty of Medicine,
Ain-Shams University
2014

Acknowledgment

Before all, Thanks to **ALLAH**

I would like to express my profound gratitude to Professor / Nasser Sadek Rezk*, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for his most valuable advises and support all through the whole work and for dedicating much of his precious time to accomplish this work.*

I am also grateful to Professor / Manal Abd El-Baky Mahmoud*, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her unique effort, considerable help, assistance and knowledge she offered me throughout the performance of this work.*

My special thanks and deep obligation to Professor / Nermin Helmy Mahmoud*, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her continuous encouragement and supervision and kind care.*

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AF	Advanced fibrosis
AFB1	Aflatoxin Bl
AFP	Alpha fetoprotein
AFP mRNA	Alpha fetoprotein mRNA
AFU	Alpha-l-fucosidase
AH	Adenomatous hyperplasia
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AR	Androgen receptor
AST	Aspartate aminotransferase
AUC	Area under the curve
bcl-2	B-cell lymphoma 2
bFGF	Basic fibroblast growth factor
CA 125	Cancer antigen 125
CBC	Complete blood count
Cdks	Cyclin-dependent kinases
CEA	Carcinoembryonic antigen
CEUS	Contrast enhanced ultrasound
CLD	Chronic liver diseases
COX-2	Cyclooxygenase -2
CpG	Cytosine phosphate guanine
CT	Computed tomography
DAB	Diaminobenzedine tetrahydrochloride
DCP	Des-gamma-carboxyprothrombin
DM	Diabetes mellitus
ECLIA	Electrochemiluminescence immunoassay
ECs	Endothelial cells
EF	Early fibrosis

EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptors
ELISA	Enzyme Linked Immunosorbant Assay
ENG	Endoglin
ER	Estrogen receptor
FN	False negatives
FNAB	Fine needle aspiration biopsy
FP	False positive
GGT	Gamma-glutamyl transferase
GGT mRNA	Gamma glutamyl transferase mRNA
GP73	Golgi protein 73
GPC3	Glypican-3
GST-Pi	Glutathione S-transferase-Pi
GSTs	Glutathione S-transferases
hTEP1	Human telomerase associated protein l
hTERT	Human telomerase reverse transcriptase
hTERT mRNA	Human telomerase reverse transcriptase mRNA
HBs Ag	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCCR	Hepatocellular carcinoma receptor
HCV	Hepatitis C virus
HDGF	Hepatoma-derived growth factor
HDV	Hepatitis D virus
HGF	Hepatocyte growth factor
HHT-1	Hereditary Hemorrhagic telangiectasia type 1
HIF-1	Hypoxia inducible factor-1
IGF II-mRNA	Insulin-like growth factor II-mRNA
IL-6	Interleukin-6
IL-8	Interleukin-8
IM	Intrahepatic metastasis

IMVD	Intratumoural microvascular density
INR	International normalised ratio
IQR	Interquartile range
LCA	Lectin lensculinaris agglutin
LD	Lactate dehydrogenase
mAb	Monoclonal antibody
MDL	Minimum detectable limit
MMPs	Matrix metalloproteinases
MOH	Ministry of Health
MPCT	Multiphasic helical computed tomography
MRI	Magnetic resonance imaging
MRN	Macroregenerative nodule
MVD	Microvascular density
NAFLD	Non alcoholic fatty liver disease
NASH	Non alcoholic steatohepatitis
NPV	Negative predictive value
PDGFR	Platelet-derived growth factor receptor
PIVKA-II	Protein induced by vitamin k absence or antagonist II
PPV	Positive predictive values
PVT	Portal vein thrombi
ROC	Receiver operating characteristic
RT-PCR	Reverse transcription polymerase chain reaction
SCCA	Squamous cell carcinoma antigen
sENG	Soluble endoglin
SD	Standard deviation
SMAD	Small mothers against decapentaplegic
TGF- β1	Transforming growth factor β1
TN	True negative
TNF- ~	Tumor necrosis factor-alpha
TNM	Tumor Node Metastasis

TP	True positives
TSGF	Tumor specific growth factor
U/S	Ultrasound
UV	Ultraviolet
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
ZP	Zona pellucida

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem worldwide. It is the fifth most common cancer and the third most common cause of cancer related death **(Lau and Lai, 2008).** Patients with hepatitis B and C related liver cirrhosis are at high risk of developing HCC **(Masatoshi, 2008).** The prognosis of patients with HCC is poor when diagnosed at an advanced stage but when diagnosed and treated at early stage the 5-year survival rate may reach up to 70-80% **(Sonia et al., 2008).** Therefore early detection of HCC is a critical goal to improve the patient outcome.

Histo-pathological examination of tumor biopsy is considered the crucial method for reliable diagnosis of HCC. However, it is mandatory to examine non-tumoral hepatic tissue to exclude or to confirm the presence of liver cirrhosis, which affects the treatment modality. Ultrasound-guided fine needle biopsy accurately diagnoses HCC in about 90% of nodules, including nodules of a very small diameter. However, malignant seedling is a recognized complication in patients with HCC and risk of tumor seedling along the needle tract has been estimated as 3% of cases **(Change et al., 2008 and Asmaa et al., 2009)**

Cross-sectional imaging techniques with computed tomography (CT) and magnetic resonance imaging (MRI) are

the most commonly used techniques to detect HCC **(Ma et al., 2008).** MRI was found to be more accurate than CT in detecting HCC and estimating the actual size of the tumor. Although its sensitivity in detecting HCC is as high as 95% in tumors larger than 2 cm, unfortunately, in tumors less than 2 cm the sensitivity is as low as 30% **(Asmaa et al., 2009).**

As regards serologic screening, alfa fetoprotein (AFP) still represents the currently used test for HCC, despite its low sensitivity which reaches only 45%. Moreover, alteration of AFP serum levels are commonly observed in cirrhotic patients **(Giannelli et al., 2005)**. In addition, development of false negative or false positive rates with AFP was as high as 30-40% for patients with small HCC **(Wei et al., 2006).** This prompted the search for recent, more reliable non-invasive biochemical markers with better sensitivity and specificity for the early diagnosis of HCC.

CD105 also known as endoglin, is a disulfide-linked homodimeric type I transmembrane glycoprotein **(Burke et al., 2010).** The expression of endoglin is elevated on the endothelial cells of healing wounds, developing embryos, inflammatory tissues, and solid tumors. Endoglin is a marker of activated endothelium, and its vascular expression is limited to proliferating cells. Recent studies identified endoglin expression in several solid tumor types **(Dallas et al., 2008).**

AIM OF THE WORK

The aim of the present study is to investigate the clinical utility of endoglin (CD 105) as a novel biomarker of hepatocellular carcinoma (HCC) and correlate its serum levels with alpha fetoprotein, as the current biochemical marker of HCC.